SUPPLEMENTAL MATERIALS

S1. Gel Methods

Ligated and unligated nanotubes were diluted to 180nM (each strand) with pure water, and denatured by mixing with equal parts of 8M urea and heating for five minutes at 90ºC. Prior to loading, a 12% polyacrylamide denaturing gel (14x10x0.15 cm) was run for 25 minutes at 30V/cm. Then 20µL of each solution was mixed with 5µL of 30% glycerol, loaded on the gel, and run at 30V/cm for 50 minutes. The gel was stained in a 100mL solution of 0.5X TBE and 1X Sybr gold for 30 minutes with gentle rocking, and imaged with a fluorimager (STORM840, Molecular Dynamics).

For quantitative analysis of the fluorescent image, NIH Image (v1.63) was used to plot intensity (averaged over the lane width) as a function of position along each lane. The total intensity for a given band was calculated from the area under a Gaussian fit to its intensity profile.

S2. Band Analysis

We calculate upper and lower bounds on the fraction of phosphorylated nicks successfully ligated, *x*. We assume that either all or none of the strands in the ligation product have adopted their lowest energy secondary structure. Secondary structures were determined using the Mfold webserver with parameters of 0.1M NaCl and 20ºC.

For one-point ligated nanotubes, the bounds on *x* are given by

$$\frac{\left(\frac{A_{63}}{3N_P + N_U}\right)}{\left(\frac{A_{26}}{26}\right)} \le \frac{x}{(1-x)+1} \le \frac{\left(\frac{A_{63}}{63}\right)}{\left(\frac{A_{26}}{26}\right)}$$

where A_{63} and A_{26} are the total fluorescence intensities from the 63 and 26 base bands, respectively. Because fluorescence is proportional to the number of bases, these intensities are divided by the number of bases per strand to yield a ratio of the number of strands in each band. For the lower bound, the number of bases per strand is weighted to take into account the three-fold brighter signal from paired bases. N_P represents the number of bases per strand involved in base-pairs, while N_U represents the number of unpaired bases. Note that N_P and N_U differ depending on which strand is initially phosphorylated because the different ligation products can have different secondary structures.

The central term is the ratio of the number of strands in the 63 and 26 base bands in terms of *x*. The numerator comes from reasoning that ligation occurs with frequency *x* and yields one 63 base strand. The denominator comes from reasoning that only one of two 26 base strands participates in the phosphorylated nick. It is only present in the 26 base band when ligation doesn't happen, which occurs with frequency (1-*x*), while the other strand is present in the 26 base lane regardless of ligation.

Similarly, for three corner ligated tubes, the bounds on x are given by

$$\frac{\left(\frac{A_{126}}{3N_P + N_U}\right)}{\left(\frac{A_{26}}{26}\right)} \le \frac{x^3}{2(1-x)^3 + 3x(1-x)^2 + x^2(1-x)} \le \frac{\left(\frac{A_{126}}{126}\right)}{\left(\frac{A_{26}}{26}\right)}$$

where the central term is derived as follows. Note that if fully ligated, the tubes would form a mesh of concatenated large and small loops. Here we use "loop" to mean the large loop consisting of strands #1, #2, #4, and #5. Prior to ligation, it is possible to retain the notion of loops, but to imagine each loop as containing four nicks. The numerator represents the fact that each 126 base strand requires three nicks on the same loop to be ligated, which happens with frequency x^3. The denominator represents the many ways of getting 26 base strands. If none of the nicks on a given loop are ligated, as occurs with frequency $(1-x)^3$, then both 26 base strands remain. There are three ways to ligate one of the nicks on a loop, each yields one 26 base strand and occurs with frequency $x(1-x)^2$. There are three ways to ligate two of the nicks on a loop, each occurs with frequency $x^2(1-x)$, but only one of them yields a 26 base strand.

S3. Melting Experiments (fluorescence microscopy)

Ligated and unligated nanotubes were diluted in 1X TAE/Mg (40mM Tris-acetate, 1mM EDTA, 12.5mM Magnesium acetate) to a final DNA concentration of 144nM (each strand). PCR tubes containing 9μL of the diluted solutions were held at a fixed temperature for 30 minutes by submersion in a temperature-regulated water bath. After removal from the water bath, the solutions were placed on ice for 10 min and 1uL of oxygen-scavenging system (OSS) was added to each solution to inhibit photobleaching[1]. Microscope samples were prepared on cleaned, bare glass, and sealed with paraffin. The excess of Mg^{++} ions in the buffer mediates binding of the nanotubes to the glass, thereby "freezing" the length distribution. Nanotubes were bound to the glass within 3 minutes of removal from the water bath. Images were captured with a CCD camera (Sensicam, Cooke) on an inverted fluorescence microscope (Olympus IX70) using a 100x oil immersion objective.

S4. Atomic Force Microscopy

AFM imaging was performed in Tapping Mode under 1X TAE/Mg buffer on a Digital Instruments Nanoscope III (Veeco) equipped with a nanoAnalytics Q-control III (Asylum Research) and a vertical engage J-scanner, using the ~9.4 kHz resonance of the narrow 100μm, 0.38 N/m force constant cantilever of an NP-S oxide-sharpened silicon nitride tip (Veeco Metrology). Samples were prepared for AFM imaging by deposition of ~5μL onto a freshly-cleaved mica surface (Ted Pella) attached by hot melt glue to a 15 mm metal puck; an additional 30μL of buffer was added to both sample and cantilever (mounted in the standard Tapping Mode fluid cell) before the sample and fluid cell were positioned in the AFM head. The tapping amplitude setpoint, after engage, was 0.2 - 0.4 volts, the drive amplitude was 100-150 millivolts, and scan rates ranged from 2-5 Hz. After acquisition, images were flattened by subtracting a low-order polynomial from each scan line, or by adjusting each scan line to match intensity histograms.

S5. Persistence Length Measurements

Nanotubes were confined to two dimensions via a thin coating of PVP gel on both glass surfaces of the microscope sample[S1]. Oxygen scavenging system and 1x TAE/Mg were added to the nanotube solutions to final DNA concentrations of 18nM (each strand). The microscope samples were sealed with epoxy, which allowed imaging for many hours without appreciable evaporation. For each nanotube imaged, 200 images were captured at1 second intervals and R^2 was averaged over all images. Nanotubes labeled with TAMRA were used for these long imaging sessions because it was found to be more stable to photobleaching than FAM.

S6. Buffer Exchange

Dialysis was performed by floating 3,500 MWCO dialysis cassettes (Pierce, BF44237) in a 2L beaker of pure water. Spin filtration was performed using 100kD cutoff spin filters (Millipore, Cat. No. 42412). Solvent exchange via ultracentrifugation was achieved by pelleting the nanotubes with a 30min spin at 14,000*g*. The supernatant was removed via pipette while the PCR tube was held near a UV transilluminator for visualization of the fluorescent pellet. The pellet was resuspended in pure water with thorough pipetting.

References:

(S1) Ekani-Nkodo, A.; Kumar, A.; Fygenson, D.K., *Phys Rev Lett* **2004**, 93, 268301.